Utilization of Wheat Germ Flour in the Processing Of Beef Sausage

By

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بسم الله الرحمن الرحيم

Dedication

This force is dedicated with great love and respect to: my father, mother, my brothers, sisters and any person who support and gave me courage to accomplish this study.

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Abstract

Beef sausage was processed by additions of different replacement levels of meat by wheat germ flour (WGF) replacement levels were : 0% (as control) 10% and 15% .The processed beef sausage were packaged in foam trays , over–wrapped with polyvinyl chloride (PVC) and stored refrigerated at 4°C ± 1 for up to 7 days. Several variables were determined using subjective and objective measurements, to evaluate the effects of replacement levels and storage periods on the quality attributes of the processed beef sausage . The measured parameters included : proximate composition, water holding capacity (WHC), pH, peroxide value (PV), storage loss, cooking loss and sensory attributes of beef sausage . The evaluation was conducted immediately after processing, three and seven days post processing day.

Results demonstrated that control sample (0% replacement level) had the lowest ($p < 0.05$), protein, fat, ash and crude fiber content . Lower scores in over all acceptability, aroma and flavor; but higher score ($p < 0.05$) in deviation from meat aroma, moisture content, PV and cooking loss%. There were no significant differences ($p > 0.05$) in fat content, ash content , crude fiber content, PV and storage loss, among the samples from the different replacement levels due to the Storage period.

Fifteen % replacement level sample had the highest ($p < 0.05$) on protein , fat, ash , crude fiber contents, overall acceptability, flavor , aroma scores and water binding capacity and Lower ($p > 0.05$) P V and pH values.

Water holding capacity is increased with the increase of replacement levels. Protein content, fat content, ash content , crude fiber content, overall acceptability score, flavor score and aroma score, were increase with the increased of replacement levels.

WGF act as binder in beef sausage production and could be a good substitute to others plant binders wich are used as meat binder or extenders.

ملخص البحث

تم تصنيع السجوك البقري بإضافة دقيق جنين القمح باستخدام نسب استبدال مختلفة لبروتين اللحم ألبقري بدقيق جنين القمح . و نسب الاستبدال هي : ا 0% (العينة الشاهد)، 10% و 15%. السجوك ألبقري المصنع، تمت تعبئته في أطباق بلاستيكية و من ثم غلفت بفلم بلاستيكي رفيع من ال Polyvinyl (PVC) chloride عديد كلوريد الفينيل، ومن ثم تم تخزين العبوات مبردة في درجة حرارة 4°م ±1 لمدة سبع أيام. تمت دراسة العديد من المتغيرات باستخدام وسائل تحاليل مختلفة لتقييم أثر نسبة الاستبدال واثر التخزين علي خصائص الجودة للسجوك ألبقري. واشتملت المتغيرات التي تم قياسها علي :تحديد محتوي البروتين، الدهن، الرطوبة، الرماد، الألياف و الكربوهيدرات، وقيمة البيروكسيد والأس الهدروجيني(pH)، و مقدرة إمساك الماء (WHC)، فاقد الطبخ، والفقد في التخزين بالإضافة إلي الصفات التذوقية لمنتج السجوك ألبقري. اجري التقيم بعد تصنيع المنتج مباشرةً وفي اليوم الثالث والسابع بعد التصنيع.

أوضحت التجارب أن العينة الشاهد (ذات نسبة الاستبدال 0%) أعطت ادني (P <0.05) محتويات للبروتين، الدهن، الرماد والألياف وتدنت(P <0.05) فيها نسبيا درجة التفضيل للصفات التذوقية بالنسبة الطعم والنكهة والتقبل الكلي وازدادت (P <0.05) نسبة الرطوبة وفاقد الطبخ فيها. لم يكن هنالك اثر محسوس (p > 0.05) للتخزين علي محتوى الدهن، محتوى الرماد، محتوى الألياف،فاقد التخزين و قيمة البيروكسيد.

نسبة الاستبدال 15% أعطت أعلا (P <0.05) محتويات للبروتين، الدهن، الرماد ،الألياف. وتحسنت (P <0.05) فيها درجة التفضيل للصفات التذوقية بالنسبة للطعم، والنكهة، والتقبل الكلي.وزادت (P <0.05) فيه مقدرة إمساك الماء و انخفضت (P <0.05) فيها قيمة البيروكسيد والرقم الهيدروجيني.

خلصت الدراسة إلي إن العينات المعاملة بإضافة دقيق جنين القمح زاد (P <0.05) فيها محتوى البروتين، الدهن، الرماد ،الألياف. وتحسنت (P <0.05) فيها درجة التفضيل للصفات التذوقية بالنسبة للطعم، والنكهة، والتقبل

الكلي.وزادت (P <0.05) فيها مقدرة إمساك الماء بزيادة نسبة الاستبدال. و انخفضت (P <0.05) قيمة البيروكسيد والرقم الهيدروجيني. كما انخفض (P <0.05) الفقد في الطبخ بزيادة نسبة استبدال بروتين اللحم ألبقري بدقيق جنين القمح.

لذا فإن دقيق جنين القمح يمكن أن يكون بديلا جيداً للبروتينات ذات المصدر النباتي والتي تستخدم في منتجات اللحوم المصنعة.

Chapter One

Introduction

Meat is that edible substance of the many edible food animals, to the trade the term is usually applied to carcase (Gracey, 1986).

Sudan has a great meat processing potentialities due to the huge Sudanese livestock resource, according to Pan African Programme for the Control of Epizootics (PACE. 2002) Sudanese livestock population for the year 2001 estimated in '000 was 38,325 cattle, 47,034 sheep, 39,952 goats and 3,203 camels.

Sausage is one of the old meat products in which fresh comminuted meat are modified by various processing methods to yield desirable organoleptic and keeping properties (FAO. 1985).

Meat and meat products are highly perishable materials so sanitation and cooling is essentials in handling, marketing and processing of meat. The sanitation in the Sudan, in general is very poor with regard to slaughtering, handling, marketing and processing of meat, except for very few meat plant and slaughter houses.

The demands for the fast meal and ready made food (a sausage is one of the major products used for this meals), is increased in the recent years in worldwide. In Sudan, the strong demands for these types of foods may be due to:

*Internal Migration from rural to urban areas for education, work, etc.

* Education, governmental and non-governmental universities and good braved schools where usually located in the big cities.

*Many Sudanese women no longer stay at home for child care or meals cooking but educated and employed in education institutions, factories and other related sectors to improve their social status.

*Added to that the external migration, many non-Sudanese labors come from other Asian and African countries to work in Sudan they consumed the fast meals because these meals are international.

Generally, meat products are widely consumed throughout the world; but unfortunately, their cost is high. To reduce this cost there is increasing interest in use of various non-meat proteins especially plant protein. Non-meat protein include vegetable protein soya beans, cereal and legume protein and are often referred to in the trade name as "meat extenders" or "meat substitutes" (Gracey, 1986).

Lin and Zayas (1987) reported that increasing cost of animal protein sources has encouraged researchers to study alternative protein sources, to be used in comminuted meat products, because of their lower formulation cost.

The objectives of this study are:

1 - to evaluate the effects of partial replacement of meat by wheat germ flour on the quality characteristics of beef sausage.

2- To evaluate the effects of storage periods on the quality characteristics of beef sausage.

Chapter Two

Literature Review

2.1. Nutritional Value and Composition of Meat

2.1.1. Nutritional Value

Food consists of all those substances which when taken into the living organism to produce energy, build tissue, or regulate the life process without any harm occurring to the organism. Meat implies edible substances from muscles, organs, and glands of the many food animals, but to the trade the term is usually applied to carcase. (Gerrard, 1977).

Meat is an excellent source of protein, iron and Vitamin B (NIIR., 2004). Nutritionally, meat is a very good source of essential amino acids, to a lesser extents, of certain minerals. Although vitamins and essential fatty acids are also present, meat also provides calories from protein, fat and limited quantities of carbohydrate (Judge *et al*., 1990).

The inorganic minerals or ash of muscle constitute a about than 1% of the content, the main element being, in order of importance: sulphur, potassium, phosphorus, sodium, chlorine, magnesium, calcium, iron and zinc. Vitamin occurs in the form of the water-soluble B vitamins along with some vitamin A (lipid soluble) and vitamin C (water soluble ascorbic acid). The level of vitamin in meat reduced by cooking, the amount depending on the temperature and time employed (Gracey, 1986).

Gerrard (1977) concluded that nutritionally the calorific value of meat is affected by the fat contents. As the % of fat increase in meat the calorific value increase also (Table 1).

Table (1) Composition of Some Common Meat:

Meat source	Waters	Protein	Fat	Carbohydrate	Kcals/100g
calf	74	18	6	-	126
Lean beast	65	16	18	-	226
Beast average	56	15	28	-	312
Lamb	55	13	31	-	331

Source: Gerrard, (1977)

2.1.2. Composition of Meat:

The chemical and biochemical contents of the muscle are affected by intrinsic and extrinsic factors. The most important intrinsic factors are species, sex, age, and anatomical location of muscle. The extrinsic factors are nutrition, fatigue, fear, pre-slaughter manipulation and environmental conditions before, during and after slaughter. Generally the composition of meat is 75% water, 18% protein, 3.5% soluble non-protein substances and 3% fat (Lawrie, 1991).

The total fat available depended up on the life weight and the degree of finish in the animal for example a very good ox might produce (25kg) from life weight 660kg. The fat should be dealt with as quickly as possible and cooled rapidly to obtain the best product.(Gerrard, 1977).

The major contribution of fat to the diet is energy or calories. This is true, because fat has 2.25 times as much energy as equal quantity of carbohydrate or protein. However, energy is not normally the limiting factor in most American diets; rather, too many calories is the more likely problem. On the other hand, limiting the intake of fat is a common method of weight control. This creates a demand for lean meat products with a low fat content. Such as boiled ham, Canadian-style bacon, Lebanon bologna, and similar products (Pearson and Gillett, 1999).

Health organizations have promoted the reduction of fat and cholesterol in obesity. Diets containing not more than 30% of their calories from fat are recommended. Many meat products with standards of identity, such as frankfurters and bologna, were regulated in the U.S to the extent that their formulation were practically mandated. Maximum fat levels were set at 30% and added water at 10% (Pearson and Gillett, 1999).

2.2. Quality Attributes of Meat:

Quality, like beauty, is a very subjective attribute which varies from country to country and region to region. Various definitions have been put forward over the years, but all have suffered from the lack of any objective approach and have generally concluded that quality meat was that for which the public was prepared to pay the highest price (Cooper and Willis, 1984).

2.2.1. Colour:

In recent consumer preference investigation in Britain it was found that some of 60% of the housewives gave colour as being the most important initial consideration in their selection of beef. color of meat is a very important consideration in the consumer perception of meat and definitely will affects marketability (Gerrard, 1977).

Basically, the chemistry of meat colour is that one pigment, myoglobin (myoglobin is complex protein similar to the blood pigment hemoglobin). With the life animal this substances will account for about 10% of total iron but during the bleeding a very high proportion of the iron is removed as hemoglobin. In the muscles from a well-bleed beast particularly all the remaining iron is accounted for myoglobin. Myoglobin function is essential for storing the oxygen with in the meat cells (Gerrard, 1977).

The color-bearing Ferro protein in the blood hemoglobin is capable of undergoing the same oxygenation reactions as the Ferro protein pigment in lean meat myoglobin, to produce well-known bright red colored meat (Anderson *et al*., 1989)

2.2.2. Tenderness:

Tenderness is the most important palatability characteristic. In general, muscles containing the least connective tissue are more tender this correlation is not always fool proof. Tenderness of meat involves physical, physiological and biochemical factors of muscles that may be broadly divided ante-mortem and post-mortem factors. The later group may further be separated into ante-rigor mortis and post-rigor mortis. A number of processor have been devised to influence these factors so that an optimum tenderness result. Naturally occurring meat proteolytic enzymes may be utilized under controlled conditions to age or tenderized meat (NIIR, 2004).

2.2.3. Juiciness:

This is a very difficult character to assess since it is related not only to the initial impression of wateriness, due to release of meat fluid, but also to the longer lasting effects of fat in the meat stimulating the salivary glands. As a result one might expect some relation between fatness and juiciness and this in the facts the case. Young animal will give juicy meat (Cooper and Willis, 1984).

The principal sources of juiciness in meat, as detected by the consumer are the intramuscular lipids and the water content, the marbling that are present also serves to enhance juiciness during the cooking process when the melted fat apparently become translated along the bands of perimysial connective tissue. This uniform distribution of lipids throughout the muscle may act as a barrier to moisture lost during cooking (Judge *et al*., 1990).

2.2.4. Firmness and Texture:

Firmness of the flesh associated with pre and post mortem treatment of cattle and may be connected with water holding capacity. Firmness does not seem to be associated with fatness and well marble carcasses are unlikely to suffer from watery muscle texture and hence coarse texture meat will be tougher to eat (Cooper and Willis, 1984).

2.2.5. Water Holding Capacity (WHC):

The water holding capacity of the meat is attribute of obvious importance. This particularly so in comminuted meat such as sausage where the structure of the tissue has been destroyed and is not longer able to prevent the release of the fluid from the protein (Lawrie, 1991).

Water holding capacity (WHC) is the ability of meat to retain its water or added water during application of external forces such as cutting, heating, grinding or processing. Many of the physical properties of meat including colour, texture and firmness of raw meat, juiciness and tenderness of cooked meat are particularly dependant on WHC (Judge *et al*., 1990).

2.3. Deterioration of Meat Quality:

A number of methods are employed throughout the meat industry to retard deterioration changes and extend the length of the acceptability period. This depends mainly on preservative method and inherent properties of specific meat items. The post-mortem changes associated with conversion of muscle to meat, and subsequent storage and hand linking are caused by microorganism (bacteria, mold and yeast), insect, indigenous enzymes naturally present in meat, exogenous enzymes (secreted by microorganisms) and physical effects (freezer burn, drip, light fading and discoloration). The microbial source includes equipment, clothing and hands of personnel, air, water, wall and doors (Judge *et al*., 1990).

2.4. Sausages :

Meat was processed as early as prehistoric times, probably drying in the sun and later by smoking over wood. Today, meat is processed with salt colour fixing ingredients, and seasonings in order to impart desired palatability traits to intact and comminuted meat products. Intact meat product includes bacon, corned beef, ham, smoked and pork hocks. Comminuted meat products include all types of sausage items (NIIR, 2004).

Meat production was increased in the last 20 years (1980-200)in worldwide for example North America meat production in the year -1980 was (15,747.5), increased to reached (19,837.6) by the year -1990 and in -2000, the production was(20,131.) thousands metric tons (NIIR. 2004).

The term sausage is derived from the Latin word (salsus) meaning salt or literally translated, refers to chopped or minced meat preserved by salting. In ancient time, the sausage mixtures were encased in animal intestines and consequently were more or less cylindrical in shape. Sausage are one of the oldest form of processed foods, their origin being lost in antiquity, it has been reported that sausages were used by the Babylonians and the Chinese 1500 B.C. (Pearson and Gillett, 1999).

2.4..1. Classification of Sausage:

Sausage may be roughly divided in to two general groups: raw sausage and heat processed sausage. According to the methods applied in their manufacture, raw sausages may further be subdivided in to two categories: fresh sausage and fermented sausages. Similarly, heat processed sausage are classified in smoked precooked sausage, emulsion-type sausages and cooked sausages.

A-Fresh sausages are made from fresh meats.

B - Fermented sausages are made from cured or uncured, fermented and often smoked meats but they are not heat processed in any way, they are divided into semidry and dry sausages.

C - Smoked precooked sausages are mostly cured, nonfermented products, their shelf life is increased by heating due to partial reduction of their moisture content; they are usually finally cooked before consumption.

D - Emulsion type sausages comprise ready-to-eat products made from comminuted and well-homogenized cured meats, fatty tissue, water and seasonings, usually smoked and slightly cooked.

E - Cooked sausages are ready-to-serve products, basically made from previously cooked fresh or exceptionally cured raw materials, subjected to final cooking after stuffing, with or without additional smoking (FAO. 1985).

2.4.2. Fat in Sausage Production:

Beef fat is a valuable raw sausage ingredient which requires special care. It easily becomes sour or rancid if improperly handled or if kept under improper conditions. Beef fat should preferably be used as fresh as possible, without freezing and storing. If however, beef fat must be stored, the storage temperature should not exceed 5^{o}C. Old or rancid fat should never be mixed with fresh fat. Old fat will simply contaminate and ruin any other fat mixed with it. The unprocessed meat sausage mass containing such fat very often appears to be entirely fresh but the finished sausage may still be of a low organoleptic value or quite inedible. The best fats for making all beef sausage are brisket fat and back fat. The white fat of not too young beef animals is preferred for sausage making. Firm white fat is associated with quality sausages (FAO. 1985).

As with meat fat are divided commercially, into two main categories of edible and inedible fats. The edible fats are derived from material from healthy animals, cleanly and carefully processed. Inedible fat recovered from the tissue of dead animal. According to melting point they divided into two classes Tallow have melting point 104^{o}F (40^{o}C) or higher, while that of the "grease" is below 104^{o}F (FAO. 1960).

2.4.3. Ice or Water in Sausage Production:

Many products would be dry and unpalatable if only the moisture contained in the meat ingredient were present in the final product. Additional water improves their tenderness and juiciness. Water added as ice also to keep product temperature down during emulsification. The water added to the burger formulations also serves to replace water that will be lost during processing operations. Thus, by adding water, the yield of finished product can be improved (Forrest *et al.*, 1975).

2.4.4. Vegetables Proteins as Meat Extenders:

There is a wide variety of non-meat products. These products are refereed to as binders or extenders and less frequently as fillers. They are added to meat formulations for one or more of the following reasons. To reduce formulation cost, to improve cooking yield, to improve slicing characteristic, to improve flavor, to increase protein content, to improve emulsion stability, to improve fat binding and water binding. (Pearson and Gillett, 1999).

2.4.4.1 Soya Bean as Meat Extenders:

Soya protein extenders are three main classes of products namely soy flour, soy protein concentrate and soy protein isolate. All three products are used in processed meat, sausages (Pearson and Gillett, 1999).

Meat patties were prepared by replacing beef partly with mixture of protein-rich plant products in such ration that each component contributed 50% of the protein in the blend (Ndupuh and Akobadu, 1984).

Samh flour is very comparable to Soya protein concentrate and could be a good substitute for it in meat product formulation (Elgasim, 1999).

2.4.4.2. Wheat Gluten:

Wheat gluten it is the protein of the flour component of wheat. Wheat gluten has power of adhesion to meat. In addition to the meat binding property of gluten, the colour of cooked gluten is darker than that of flour (NIIR, 2004).

2.4.4.3. Wheat Germ:

The germ in the cereal is that component partly responsible for the plant growth or development (Badi and Monawar, 1987).

Wheat germ is the embryo of the wheat kernel. It is separated from wheat being milled for flour (Canasambanadam and Zayas, 1992).

Quisenberry and Reitz (1967) reported that the wheat grain has average composition 85% endosperm, 12% bran and 3% germ, while Egan *et al*. (1981) reported that the average composition is 85% endosperm, 12.5% bran and 2.5%. germ

2.4.4.3.1 Nutritional Value and Composition of WGF:

Germ constitutes about 2.5% of the grain weight and comprises minimal amount of protein, but greatest share of fat, vitamins especially tocopherols (Anon, 1987).

Shurpalekar and Rao (1977) showed that wheat germ contained three times as much protein of high biological value, seven times as much sugar and six times as much mineral compared with flours from endosperm. In addition wheat germ is considered the richest known source of plant origin of vitamin E (α-tocopherol) and a rich source of vitamin B-group.

Amino acid composition of wheat germ (g/100g) protein are Alanin 7, Arginin 8.96, Aspartic acid 10,2 cystine 0.66, glutamic acid 15.45, glycine 6.54, histidine 2.63, isoleucine 3.91, leucine 6.79, lysine 7.76, methionine 1.88, phenyl alanine 4.07, proline 4.37, serine 4.62, threonine 4.82, tyrosine 3.12 and valin 5.65 (Pomeranz *et al*., 1970)

Wheat germ contained 25-30% protein, 8-10% fat 47% carbohydrate and supplied body with 374 kcal/ 100g. U.S.D.A. (2005)

2.4.4.3.2. Stabilization of Wheat Germ:

Sherwood, *et al*. (1933) studied the stabilization of wheat germs packed in vacuum and inert gas such as N_2 and Co_2 at different temperature considering acidity changes as well as organoleptic as quality measure.

Vacuums packed germs were found to be better than those packed in inert atmosphere. The rate of increase of free fatty acids (FFA) was primarily dependent on temperature of storage. At 25^oC, the average daily increase in FFA vale was in the order of 8-10 times greater than at -10^oC.

Fretzdroff *et al*. (1991) showed that the increase in the amount of FFA of stored wheat germs increased with the increase of the amount of wheat bran contaminating the germ fraction. That increase in FFA value was found to be correlated with the rise of temperature.

2.4.5. Seasoning and Curing:

Salt (sodium chloride) serves four functions in sausage: dissolves in water to form a brine which acts to retard microbial growth; it aids in solubilizing the myosin-type proteins of comminuted muscle for emulsifying the fat in emulsion sausages; it increase the water holding capacity, it contributes to basic taste characteristics and enhances the natural meat flavor (Pearson and Gillett, 1999).

Sugar in the curing of meat is common. However, in most instances, sugar is used as adjunct to provide flavor, mask the salt flavor or to provide a reservoir for an acid-forming substance (Pearson and Gillett, 1999).

Modern meat curing technology has provided efficient methods to accelerate and stabilized cured color. Ascorbic acid and its derivatives are particularly effect in rapid cured color development and its stabilization (Endel, 1977).

Nitrite and/or nitrate:

The function of nitrite in meat curing is fourfold: (1) to stabilize the color of the lean tissues, (2) to contribute to the characteristic flavor of cured meat, (3) to inhibit growth of a number of food poisoning and spoilage microorganisms, and (4) to retard development of rancidity. Although color stabilization was originally the primary purpose of adding nitrite to curing mixtures, its effects on flavor and inhibition of bacterial growth are even more important (Pearson and Gillett, 1999).

2.4.6 Emulsion Stability:

Emulsion stability means reduced fat and water loss absorbed upon cooking this result improve cooking yield and also in better flavor and color. Emulsion preparation is a critical step in sausage processing technology; the objective is to promote the formation of stable emulsion free of fat separation, jelly pockets, fat caps and surface grease (Endel, 1977).

Consumers of cooked, cured meat emulsion products associate a bright pink or red color as being a normal surface characteristic for products such as frankfurters, bolonas, salamis, and the like. This color is usually developed during cooking, curing or processing the meat emulsion. Sausage emulsion containing ascorbic acid may be heated immediately and uniform cured color result throughout the product. Ascorbic acid apparently first reduces metomyoglobin to myoglobin and second reacts with nitrate to produce nitric oxide more efficiently (Endel, 1977).

2.4.7. Casing:

Casings are used to make most sausage as well as some other processed meat. They determine sausage size and shapes (Pearson and Gillett, 1999).

Animal casing / product in animal casing (animal intestine), cost more, but certain products require them. Animal casing are usually edible so that the consumer generally eat the casing along with products. However, animal casings are less uniform in size tend to be more fragile, and require more care in stuffing. The high cost of animal casings couple with a slower rate of stuffing contribute to a higher cost for product in this type of casing (Pearson and Gillett, 1999).

Celluloses Casings include those from cotton bags and those derived from processed cotton linters or wood pulp (Pearson and Gillett, 1999).

2.5. Meat and Meat Product Packaging Films:

The main criteria in assessment of packaging film include the fallowing: thickness, clarity, cost, strength, degree and gases, that characteristic, for shrinking, and the lower limits for cold storage. In addition, with meat products its resistance to grease is of importance (Pearson and Gillett, 1999).

2.6. Storage of Meat and Meat Products:

The underlying principle of all food preserving methods, then, is the creation of conditions unfavorable to the growth or survival of spoilage organism by for example, extreme heat or cold, deprivation of water and some times oxygen, excess of saltiness or increased acidity (Gracey, 1986).

In case of frozen meat growth of bacteria is inhibited and event at chilling temperature 28 to 30^{o}F or -2 to -1^{o}C their growth reduce to extend considerably the commercial life of the product (Gerrard, 1977).

Gerrard (1977) found that the refrigeration temperature retarded the bacterial developed on the meat surfaces as it shown in fig (1)

The rate of cooling will depend upon the temperature difference between the beef and the cold store, the depth of the flesh and particularly the thickness of the fat on the surface, as fat is very poor conductor of heat, and finally the circulation of the air and its humidity (Gerrard, 1977).

It was early discovered that proper air circulation refrigeration if meat come in contact with ice, discolor and spoilage might result (Jensen, 1949).

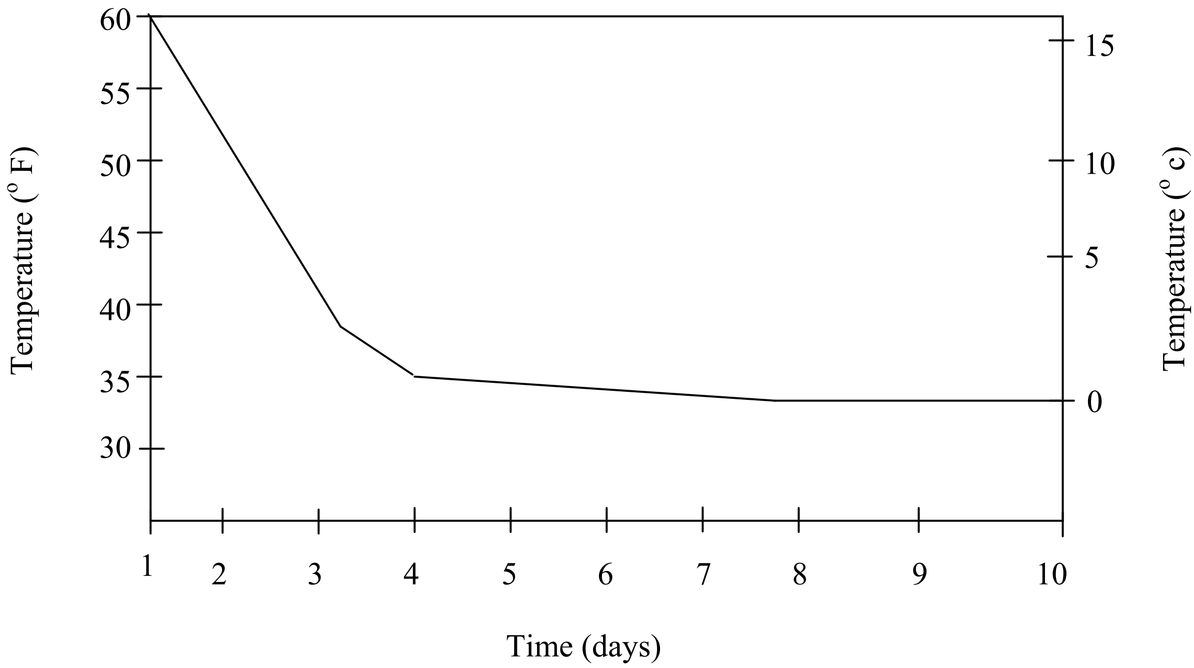

Fig (1) Temperature Against Time for Bacterial Slime to Develop on Meat Surfaces Exposed Under Moist Conditions.

Source Gerrard (1977)

2.7. Microbial Effects:

The microorganisms on carcass meat originate from two main sources, e.g. the contamination on skin, hand and organisms from the intestinal tract which is occasionally punctured during evisceration (Mulder, 1996).

Meat is frequently incriminated in *staphylococcal* to the absorption of staphylococcal extraction preformed in food (Lior *et al*., 2003).

Chapter Three

Materials and Methods

3.1. Materials.

3.1.1. Food Materials.

Meat loins and round were obtained from Animal Production Research Center Kuku. The beef meat was stored frozen at -11 ±1 ºC in freezer at Regional Training Center for Meat Quality, Grading and Meat Technology, Elkadaro.

Wheat germ was obtained from Seen flour mills stored frozen. Spices, salt and sugar were obtained from local market of Khartoum North.

The additional fat needed in the formulation was obtained from the local market. Uniform rendered fat free of protein was used.

3.1.2. Chemicals and Reagents.

Chemicals and reagent used were brought from the central lab stores of Khartoum University, sodium nitrite and ascorbic acid, were obtained from Looly Company, Khartoum.

3.1.3. Casings.

Cellulose casings 23 mm in diameter were obtained from Looly Company, Khartoum.

3.2. Methods

3.2.1. Raw Materials Preparation:

3.2.1.1. Meat Preparation:

Stored beef was allowed to thaw and sliced then ground through a 0.75 In, plate using a meat grinder. Ground beef was stored refrigerated at 4 ºC ±1, for about 20 hr, a sample was taken to be analyses for protein fat and moisture content following A.O.A.C. Method (1995) Table (3).

[

3.2.1.2.Wheat Germ Preparation.

Stored wheat germ was ground, to form wheat germ flour (WGF). Then a sample was taken and analyzed for protein, fat and moisture content, following A.O.A.C. method (1995) Table (3).

3.2.2. Calculation for Sausage Formulation:

The experiment designed to produce sausage with the following specification, protein 15%, fat 20% moisture ,58.3% added starch 4.7%, salt 1.5%, and spices 0.5%.

Three batches with three replacements of meat by wheat germ were used every batch weight 2000g

Therefore protein required $= \frac{15 \times 2000}{100} = 300g$

Fat required $= \frac{20 \times 2000}{100} = 400g$

Water required $= \frac{58.3 \times 2000}{100} = 1166g$

Starch required $= \frac{4.7 \times 2000}{100} = 94g$

Salt required $= \frac{1.5 \times 200}{100} = 30g$

Spices required $= \frac{0.5 \times 200}{100} = 10g$

Sodium nitrite 100ppm.

Vitamin C 0.466g /kg

Table (2): Sausage Formula.

components	%	Weight in grams
Protein	15	300
Fat	20	400
Starch	4.7	94
Water	58.3	1160
Salt	1.5	30
spices	0.5	10

3.2.2.1. First Replacement Level

Wheat germ 0% so the required protein was 100% from meat beef therefore beef require $= \frac{300 \times 100}{22.6} = 1327.43$

Fat in 1327.43g beef $= \frac{3.2 \times 1327.43}{100} = 42.48$

Fat to be added $= 400 - 42.48 = 357.52$

Moisture in 1327.43g beef $= \frac{71 \times 1327.43}{100} = 942.47$

Moisture from starch = 6

Total moisture =948.47

Moisture to be added = 1166 - 948.47 = 217.53

Required sodium nitrite = 0.13g

Required Vitamin C = 0.62g

3.2.2.2. Second Replacement Level.

Wheat germ 10% so the required protein was 90% from beef and 10% from wheat germ

There for beef required $= \frac{300 \times 90}{22.6} = 1194.7$

Wheat germ required $= \frac{300 \times 10}{27.2} = 110.3$

Fat in 1194.7g beef $= \frac{3.2 \times 1194.7}{100} = 38.23$

Fat in 110.3 wheat germ $= \frac{6 \times 110.3}{100} = 6.62$

Total fat = 44.85

Fat to be added = 400 – 44.85 = 355.15

Moisture in 1194.7g beef $= \frac{71 \times 1194.7}{100} = 848.24$

Moisture in 110.3g wheat germ $= \frac{10.35 \times 110.3}{100} = 11.42$

Moisture in 100g starch = 6

Total moisture = 865.66

Moisture to be added = 1166 - 865.66 = 300.34

Sodium nitrite to be added = 0.12g

Vitamin C to be added= 0.56g

3.2.2.3. Third Replacement

Wheat germ 15% so the required protein in 85% from beef 15% from wheat germ.

Therefore beef required $= \frac{300 \times 85}{22.6} = 112.32$

Wheat germ required $= \frac{300 \times 15}{27.2} = 165.44$

Fat in 1128.32g beef $= \frac{3.2 \times 1128.32}{100} = 36.11$

Fat in 165.44g wheat germ $= \frac{6 \times 165.44}{100} = 9.95$

Fat to be added $= 400 - 46.04 = 353.96$

Moisture in 1128.32g beef $= \frac{71 \times 1128.32}{100} = 801.11$

Moisture in 165.44g wheat germ $= \frac{10.35 \times 165.44}{100} = 17.12$

Moisture in 100g starch = 6.

Total moisture = 824.23

Moisture to be added =1166 -824.23 = 341.8

Sodium nitrate = 0.11g

Vitamin C to be added = 0.52g

Table (3): proximate analysis and pH of beef meat and wheat germ.

Material	Protein%	Fat%	Moisture content%	Ash %	Cured Fiber %	carbohydrate	pH
Beef meat	22.6	3.2	71	0.98	-	0.3	6.29
Wheat germ	27.2	9.3	10.35	2.17	2.53	48.3	6.17

Table (4): Sausage formulation for all treatments.

Ingredient	Replacement level of meat*		
	0% protein	10% protein	15% protein
Beef meat	1327.43	1194.7	1128.32
Wheat germ	-	110.3	165.44
Starch	94	94	94100
Fat	357.52	355.15	94
Water	217.53	300.34	341.3
Salt	30	30	30
Sugar	10	10	10
Black pepper	3	3	3
Nutmeg	2	2	2
Cinnamon	2	2	2
Garlic	2	2	2
Sodium nitrite	0.13	0.12	0.11
Vitamin C	0.62	0.55	0.52

- Replacement level of meat by wheat germ on the protein to protein basses

3.2.3. Sausage Preparation

Minced meat, salt, sugar, minced fat, spices, vitamin C, sodium nitrate and half of calculated ice water were introduced to a Hobart Chopper; the Chopper was then started for about 4 min.

The added materials were dispersed uniformly. Then the ground wheat germ, starch were added together with the remainder of the calculated water.

The entire mass was chopped for about 5 min. then transferred to manual stuffer to be stuffed into cellulose casing of 23 mm in diameter and linked at lengths of 15cm. The framed sausage were heated in water at 98 ºC for a bout 40 min, fallowed by immediate cooling in ice water, for 15 min. The cooled processed sausage was peeled and packed in foam trays over-wrapped with polyvinyl chloride (PVC) and stored refrigerated for up to 7 days. the WGF replacement levels in beef sausage formulation and processing were performed following the same procedures explained above.

3.2.4. Method of Analysis

Sausage were assessed at 0 day (i-e immediately after processing)after three and seven days post processing.

3.2.4.1. Moisture Content:

The moisture content was determined according to the method of A.O.A.C. (1995). Calculated as shown below:

$$\text{Moisture content \%} = \frac{(w1-w2) \times 100}{w1}$$

Where:

w1 = original weight of sample.

W2 = weight of sample after drying.

3.2.4.2. Protein content:

The protein content of the samples was determined by the micro-kjedahl technique according to the A.O.A.C. method (1995) 0.2 g of sample was weighed accurately into micro-kjedahl flask, two hundreds milligrams of catalyst mixture and 3.5ml of concentrated sulphuric acid were added, the sample content were heated on an electric heater for 2hr and cooled, then the contents were placed into the distillation apparatus. Twenty milliliters of 40% NaoH were added the ammonia evolved was received in 10ml of 2% boric acid solution. The trapped ammonia was titrated against HCl (0.02N) using universal indicator (methyl red + brooms cresol green), the total nitrogen and protein were calculated using the following formula:

$$N\% = \frac{\text{volume of HCl} \times N \times 14 \times 100}{\text{Weight of sample} \times 1000}$$

$$P\% = N\% \times 6.25$$

Where:

N% = crude nitrogen.

P% = crude protein.

N = normality of HCl.

14 = equivalent weight of nitrogen.

3.2.4.3. Fat Content.

Total fat was determined according to the A.O.A.C method (1995). Two grams sample was extracted with petroleum ether BP 60-80 ^{o}c for 8hr. in soxhlet apparatus. The fat content was calculated according to the following equation.

$$\text{Fat\%} = \frac{(w2 - w1) \times 100}{\text{Weight of sample}}$$

Where:

W1 = weight of empty flask

W2 = weight of flask with oil

3.2.4.4. Ash content:

The ash content of sample was measured according to the A.O.A.C. method (1995) using muffle furnace (model tipoforno ZA No. 18203 Gef Ran 1001), two grams of sample was weighed into porcelain crucible and placed in a temperature controlled furnace controlled furnace at 600^{o}c for complete ashing, the crucible with ash was transferred directly to a desiccators, cooled, weight and calculated as percent of original weight of sample.

$$\text{Ash content (\%)} = \frac{(w1 - w2) \times 100}{\text{Sample weight}}$$

Where:

W1 = weight of crucible with ash.

W2 = weight of empty crucible.

3.2.4.5. Crude Fiber.

The crude fiber content was carried out by the method of A.O.A.C (1995) two grams of dried and defatted sample, were transferred to a 600ml beaker with a few anti-bombing granules. The sample was digested with 200 ml of 0.255 N sulphuric acids for exactly 30minutes, and the beaker was periodically swirled. The contents were removed and filtered through butcher funnel, and washed with boiling water. The digestion was repeated using 200ml of 0.313N sodium hydroxide for 30minutes, and treated similarly as above. After that the fiber was washed with 1% hydrochloric acid to neutralize the sodium hydroxide and then rinsed with distill water. after the last washing dish , and dried in an oven at 103 ^{o}C for one hour then cooled and weighted .the dried residue was ignited in muffle furnace at 500^{o}c over night , cooled and weight . The crude fiber was calculated using the following equation.

$$CF\% = \frac{(w1-w2) \times 100}{Ws}$$

Where:

CF% = crude fiber.

WS = weight of sample.

W1 = weight of crucible with sample.

W2 = weight of crucible with ashed sample.

3.2.4.6. Carbohydrate Content:

The total carbohydrates were calculated by difference according to A.O.A.C (1995) using formula:

Total CHO = 100-(moisture% + fat% + protein% + ash %).

3.2.4.7. Peroxide Value (PV):

Peroxide value PV of oil indicates not only the extent of over all oxidation but also resistance of oil rancidity.

To PV of the oil sample was determined according to A.O.A.C. (1995). One gram of extracted oil was accurately weight into 250ml conical flask. Thirty ml of a mixture of glacial acetic acid and chloroform (3-2) were added and the solution was gently dissolving the oil. one ml of saturated solution of potassium iodide was added. The flask was quickly shaken for 1min and kept away from the light for exactly 5 min.

Then 75ml of distilled water was added and the librated iodine was titrated with accurately standardized solution of sodium thiosulphate 0.01N using 1% starch solution as an indicator.

Peroxide value was estimated as ml equivalent of active oxygen per kilogram. I.U.P.A.C. (1987).

Duplicate determination were carried out together with blank test.

Peroxide value was determined by the following equation;

$$PV = \frac{(A - B) \times N \times 1000}{S}$$

Where:

B = reading of blank mill

A = reading of sample mil

S = weight of sample in gram

N = normality of sodium thiosulphate.

3.2.4.8. pH Measurement:

pH was measured similar to that of Herbert (1980). Ten gram of the sample was placed in a blender gar and 100 ml of distilled water were added the mixture was blended at high speed for 1 min, the pH of the mixture was measured by using pH meter. This has been calibrated with two standard buffers (6.8 and 4.0).

3.2.4.9. Water holding capacity(WHC):

Water holding capacity was measured similar to that of Backer *et al*(1968) one gram of cooked sausage was placed between two pieces of the nylon cloth (to allow separation of the meat from the filter paper) which in turn was placed between two filter (whatman No11) the whole system was placed between glassy plates firmly for two minutes the pressure was maintained sufficiently high and constant. The pressed meat was removed and weighed. WHC was express as the following equation;

Water holding capacity =100 -water index

$$\text{Water index} = \frac{\text{loss in weight} \times 100}{\text{Original weight}}$$

3.2.4.10. Storage Loss

Sample was taken from processed sausage and weighed at 0 day then stored frozen at freezer and weighed again at third and seventh day.

$$\text{Storage loss \%} = \frac{\text{Weight loss} \times 100}{\text{Weight of sample before storage.}}$$

3.2.4.11. Cooking Loss

Beef sausage samples was deep fried in cotton seed oil the cooking loss was calculated as follows:

$$\text{Cooking loss \%} = \frac{\text{Weight loss} \times 100}{\text{Original weight}}$$

3.2.4.12. Sensory Evaluation:

Ten member sensory panel consisting of M.Sc. And B.Sc. student of food science and technology Department, Faculty of Agriculture, University of Khartoum, semi-trained according to the procedure of Cross *et al*. (1978). The panel evaluated the cooked sausage sample with the different treatment for juiciness, tenderness, test, odor, differential from meat taste and over all acceptability. By mean of the scale (7=extremely like, 1=extremely dislike), Fig(3).

Panelists received samples which were randomly numbered. Water at room temperature was made available to panel for cleaning the palate between the tested samples. Plate(4)

3.2.4.13. Statistical Analysis:

The data collected from the different treatments was subjected to analysis of variance and whenever appropriate the mean separation procedure of Duncan was employed (Steel and Torrie. 1980). The SAS program (SAS, 1988). was used to perform the general linear model (GLM) analysis.

Chapter Four
Results and Discussions

4.1. Proximate Composition:

Fifteen % replacement level of meat by (WGF) significantly ($p<0.05$) reduced the moisture content (Table 5) probably due to increase of solid content . There was no significant ($p>0.05$) difference between control (0%) and 10% replacement levels in moisture content . Moisture content decreased with increase of storage period and this may be due to evaporation of moisture this result as substantiated by the fact that water in muscles exists in three forms: bounds , immobilized and free water , and free water is held by weak surface forces , As water has ability to transform from solid to vapors by sublimation. (Judge *et al*., 1990) .

Storage period within each treatments levels groups had no effect ($p>0.05$) on fat , ash , and crude fiber Table (5) . Increase of replacement level significantly ($p<0.05$) increase protein, fat, ash and crude fiber. (Table 5) that my be due to relatively high protein, fat, ash, and crude fiber of the raw WGF. (Table 3) when compared with raw beef .

The WGF replacements levels are significantly ($p<0.05$) different in carbohydrate content Table(5), carbohydrate decreased with the increased of replacement levels, the control sample had relatively the highest ($p<0.05$) value of carbohydrate content. Such a increase may be due to decrease of fat content which caused by forming of greasy surface and fat caps on the control sample this lead to loss of the fat during peeling and packaging that probably due to absents of binder,

carbohydrate content calculated by subtractions, 100-(moisture% +protein% +fat% +ash%), so decrease in any one of moisture, protein, fat and ash. Definitely will increase carbohydrate content. In 10% and 15% WGF replacements levels forming of greasy surface not observed and fat caps rarely observed in 10% WGF replacements level that my be due to present of WGF which act as binder, Pearson and Gillett (1999) showed that plant protein used as binders and extender because they absorb large amount of water and when water added became sticky causing the ground meat to adhere to each other. the later characteristic give name of binders.

4.4. Peroxide value (P.V):

The peroxide value (P.V) of Skinless beef sausage was slightly decreased ($p<0.05$) with addition of WGF Table (6) . 15% replacement level of meat by (WGF) samples had relatively the lowest P.V (Table 6) .

The decreased P.V. with increased addition of WGF may be due to vitamin E. (tocopherols) which are abundant in wheat germ Anon , (1987) . reported that germ constitutes about 2.5% of grain weight and comprises minimal amount of protein ; but greatest share of fat , vitamins especially tocopherols. Cerrard (1977) reported that vitamin E is abundant in oil obtained from wheat germ . Vitamin E act as natural antioxidant. Rumsey (2003) reported that in lipid peroxidation, the unsaturated fatty acids undergo a loss of hydrogen, resulting in the formation of a free radical at the site of unsaturation. If the feed material in which this reaction is taking place does not contain vitamin E or some other effective antioxidant, the free radical is quickly converted to a fatty acid peroxide free radical and finally to a fatty acid hydroperoxide.

Table (6) Storage loss and peroxide value of Beef sausage with or without WGF store frozen for up to 7 days.

Treatment	Storage period	Storage loss %	Peroxide value PV
(control) 0%	3	0.29^{aB} ± (0.03)	2.41^{aA} ± (0.02)
	7	0.39^{aB} ±(0.03)	2.55^{aA} ±(0.12)
WGF 10 %	3	0.35^{bA} ±(0.03)	2.19^{bB} ±(0.01)
	7	0.43^{bA} ±(0.03)	2.024^{bB} ±(0.07)
WGF 15%	3	0.27^{cC} ±(0.05)	2.06^{cC} ±(0.08)
	7	0.33^{cC} ±(0.30)	2.21^{cC} ±(0.60)

n = 3

a-c = means in the same column for the storage period bearing different small letters are Significantly different ($p < 0.05$)

A – C = means in the same column within each treatment group bearing different capital letters ($p < 0.05$).

Mistumoto *et al*. (1991) when studied the effect of vitamin E and C in improving pigment and lipid stability , found that vitamin E treatment reduced pigment and lipid oxidation compared to control samples . The result of Table (7) indicate that the storage periods (3 and 7 days) had no effect ($p>0.05$) on PV for the control (0%), 10% and 15% WGF replacement samples. The slight increase in PV of samples may be due to lipid oxidation, (Table 6). Jude *et al*. (1990) Reported that reaction of oxidative rancidity could continue slowly even in the frozen state

4.2. pH measurement:

As shown in Table (7) there was no significant difference ($p>0.05$) between 0% and 10% treatment in pH, for the 15% WGF replacement level there were significant decrease ($p<0.05$) and that may be due to relatively low pH of raw WGF (6.17), Generally , the rang of pH in all samples from the different treatments so acceptable (6.06 – 6.22) .

Gerrard (1977) reported that the meat from freshly killed cattle will usually have pH 6.5 to 6.8 (slightly acid); but its fall to lowest level , around 5.5 with a pH of 6 its considered to be of good durability , 6.4 is classified as of , insufficient durability ; whilst meat with pH reading of over 6.5 are regarded as evidence of poor keeping quality.

pH had significantly ($p<0.05$) decreased with increase of storage period for replacement levels 10% and 15% samples. in control sample (0%) there where no significant different ($p>0.05$)between 0 and 3 days storage period ; but the pH significantly ($p<0.05$) decreased after 7 days storage period .

4.3. Water holding capacity (WHC):

Water holding capacity is the ability of meat to retain its water or added water during application of external forces such as cutting , heating , grinding or pressing (Judge *et al*., 1990) .

The WHC of skinless sausage from the different treatments was given in Table (7) for all treatment samples there were significant differences ($p<0.05$) . The control sample (0%) had relatively the lowest WHC and 15% had highest WHC . WHC of skinless sausage slightly decreased ($p<0.05$) with the increased of storage period for 3 and 7 days ;but there were no significant different ($p>0.05$) among 0 and 3 days of storage period .That may be due to slightly decreased of the pH value of these samples . Varnam and Sutherland (1995). Reported that the highest water index of samples could be due to fact that these samples have lower pH values. Lower pH values may decrease water holding capacity due to protein denaturation or due to muscle protein reaching their isoelectric point. Serdaroglu and Degirmencioglu (2004) indicated that corn flour at 2 and 4% level increase moisture retention in treated meat ball samples.

4.5. Storage Loss:

At any one of the storage period tested (i.e. 3 and 7 days), the 15% WGF replacement level in the processed beef sausage tended to have lower storage losses than the control (0%) or 10% WGF replacement level samples Table (6). Numerically the difference in the storage losses between the control and 10% WGF replacement level, was so small, yet later had highest ($p < 0.05$) storage loss value at any one of the storage period tested. With in each WGF replacement level tested the storage loss of beef sausage tended to increase with the increase of the storage period i.e value on the 7^{th} days of storage period were always higher than their corresponding values on the 3^{rd} day of storage. Elgasim and Alwesali (2000) reported that addition of non meat proten such as whey or soy protein to formulate meat product has been widely practiced by meat processors. However, other plant proteins such as oat, corn germ, wheat germ protein and samh flour were found able to provide functional properties and were recommended in the processing of meat product.

The slight increase in storage loss with increase of storage period that may be due to evaporation of moisture and this agrees with the finding of Varnam and Sutherland (1995), who reported that generally the result indicated that moisture content of all samples decreased after storage due to evaporation loss commonly observed during storage at two different temperature, like the loss occurs as result of evaporation of the water from meat surface, when brought from a cold store into ordinary room temperature . An increase of The storage loss, lead to decrease of moisture content.

4.6. Cooking loss :

Fig. (2) depict changes in the cooking loss of beef sausage extended with different levels of WGF. Generally cooking loss of beef sausage was increase related with WGF replacement level. i.e. decreases with the increase in WGF level. Obviously the 15% WGF replacement level had the lowest cooking loss (12.9% v. s 17.04% and 13.85%). Among the there treatment (0% , 10% and 15% WGF replacement levels), Cnanasambandam and Zayas (1994) reported that meat batters containing wheat germ protein flour had lower cooking losses and lower

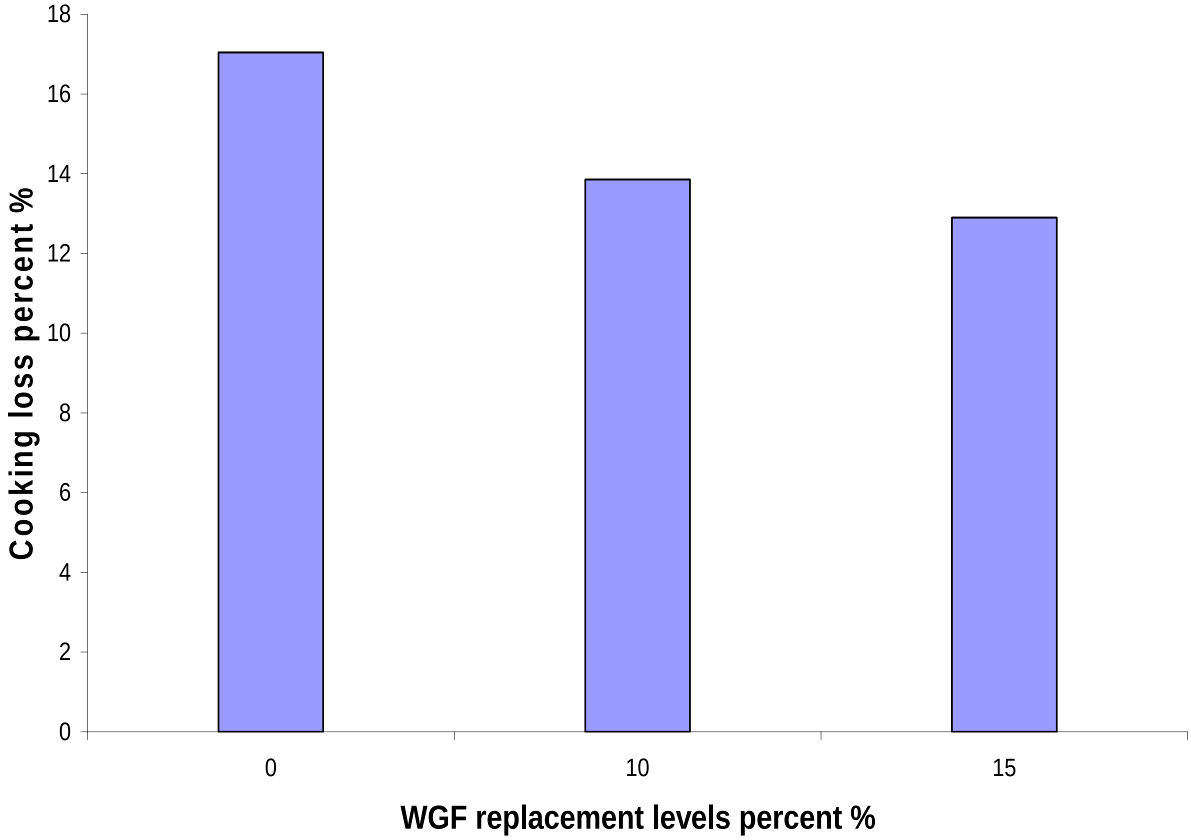

Fig.2 Cooking loss of beef sausage extended with WGF

percent water . Also the increase of WHC. Due to increase protein tended to decrease the cooking loss . Anjaneyulu *et al.* (1991) reported that this increase was in consistence's with the fact that an increase in water holding capacity (WHC) due to protein addition reduced the insignificant drop in cooking losses .

The increase of WHC during cooking my be due to the degradation of protein . Lowrie (1990) reported that the heat degradation of protein increase the concentration of peptides and amino acid and result in increase intracellular osmotic pressure and this tend to increase water holding capacity.

4.7. Sensory evaluation :

The sensory evaluation of beef sausage extended with three replacement level is shown in Table (8), the control samples scored high values in deviation from meat aroma that could be due to flavor of wheat germ flour . According to Ganasambandam and Zayas (1992) aroma and flavor are probably the most important attributes that influence the sensory properties of comminuted meat product extended with non meat protein additives.

Fifteen % replacement levels had the highest score in: aroma, flavor, Juiciness, tenderness and over all acceptability among the treatment. Generally, it was relatively similar to control sample in juiciness and deviation from meat aroma , and these agrees with the finding of Canasambanadam and zayas (1996) a trained panel found suggested an effect due to increasing lvels of wheat germ protein flour on aroma and flavor of frankfurters .

10% replacement level sample usually, scores higher than 0% and less than 15% in: aroma, flavor, deviation from meat aroma, Juiciness, tenderness and over all acceptability.

The relative high scores of tenderness and Juiciness in the sample with replacement levels 15% may be due to high water binding of these samples. Judge *et al*. (1990) indicated that many of the physical property of meat include color, texture and firmness of raw meat, Juiciness and tenderness of cooked meat are partially dependent on W H C. And mention that the portion of water present in free form and the ability of meat to bind water and factors that increase this ability will increase juiciness.

Table (8): Sensory characteristic of cooked beef

Independent variables	Replacement levels of meat by WGF			S.E
	0%	10%	15%	
Taste	4.5	4.95	5.6	± (0.34)
Order	4.6	5.05	5.65	± (0.3)
Variation from meat taste	5.20	4.60	5.15	± (0.19)
Juiciness	5.15	4.85	5.37	± (0.15)
Tenderness	5.20	5.35	5.85	± (0.2)
Overall acceptability	4.85	5.00	6.10	±(0.39)

1-7 point scale where 1 = extremely dislike and 7 = extremely like

Conclusions

Addition of wheat germ flour (WGF) to the beef sausage formula improve the proximate composition by increasing ash and crude fiber contents, also made the product more palatable to the panelists.

On the other hand utilization of WGF in processing of beef usage lead to a significant increase in water index with the increase of replacement levels of beef meat by WGF; but PV decrease with the increase of replacement levels.

Utilization of WGF in processing of beef sausage decrease cooking loss and storage loss.

Recommendations

It is recommended that use of WGF in 15% replacement level in manufacturing of comminuted meat products, to enhance their quality characteristics .

Further research is needed.

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Plate1Sample of Wheat Germ

Appendix 1

Sensory Evaluation Form

There are three types of beef sausage. Please evaluate, Aroma, Flavor, Deviation from meat aroma, Juiciness, tenderness, and overall acceptability. Using scores as fallows:

7 = Extremity like

6 = moderately like

5 =Like

4 = slightly like

3 = slightly dislike

2 = dislike

1 = Extremity dislike

If you have any question. Pleas ask

samples	A	B	C
Aroma			
Flavor			
Deviation from meaty aroma			
Juiciness			
Tenderness			
Overall acceptability			

Table (5) Proximate Composition of Beef Sausage.

Treatment	Storage period (days)	Moisture %	Protein %	Fat%	Ash%	Crude fiber %	Carbohydrate
Control 0%	0	59.15 aA ± (0.13)	14.09 aB ± (0.31)	16.49cC ± (0.31)	1.70bB ± (0.95)	0.58cC ± (0.06)	7.39bA ± (0.95)
	3	57.56 bA ± (0.47)	14.47 bB ± (0.21)	16.53 cC ± (0.28)	1.76bB ± (0.20)	0.59cC ± (0.04)	8.49 abA ± (0.62)
	7	57.22 cA ± (0.8)	15.05 aB ± (0.25)	16.19cC ± (0.19)	1.71bB ± (0.11)	0.61cC ± (0.04)	8.21aA ± (0.32)
WHF 10%	0	58.73 aA ± (0.08)	14.7 bB ± (0.5)	17.32 bB ± (0.49)	1.85aA ± (0.14)	0.63bB ± (0.05)	6.10bB ±(0.04)
	3	58.15 bA ± (0.25)	15.25 bB ± (0.05)	17.55bB ± (0.37)	1.96aA ± (0.15)	.065 bB ± (0.04)	5.92 bB ± (0.20)
	7	56.75 cA ± (0.45)	15.9 aB ± (0.4)	17.92 bB ± (0.14)	1.93 aA ± (0.07)	0.68 bB ± (0.06)	6.14aB ± (0.04)
WGF 15 %	0	57.63 aB ± (0.64)	15.65 bA ± (0.35)	17.84aA ± (0.59)	1.87 aA ± (0.12)	0.75 aA ± (0.02)	5.51bC ± (0.17)
	3	57.58 bB ± (052)	15.48 bA ± (0.88)	17.86aA ± (0.19)	1.97aA ± (0.08)	0.78 aA ± (0.02)	5.59 abC ± (0.14)
	7	56.8 cB ± (.05)	15.24aA ± (0.04)	18.32aA ± (0.55)	2.05aA ± (0.15)	0.78aA ± (0.05)	5.95 aC ± (0.11)

n = 3

a-c= means in the same column for the storage period bearing different small letters are:
Significantly different ($p < 0.05$)

A – C =means in the same column within each treatment group bearing different capital letters are:

Significantly different ($p < 0.05$).

Table (7): pH and WHC of Beef Sausage .

Treatment	Storage period (days)	WHC	pH
Control l 0%	0	62.03 aC ± (0.42)	6.22 aA ±(.010)
	3	59.55 aC ± (0.45)	6.18 aA ±(0.02)
	7	59.40 bC ± (0.30)	6.16 bA ±(0.02)
WGF 10%	0	76.55 aB ± (1.73)	6.21 aA ± (0.02)
	3	76.02 aB ± (0.22)	6.20 bA ± (0.01)
	7	75.94 bB ± (0.18)	6.19 cA ± (0.07)
WGF 15 %	0	79.78 aA ± (0.90)	6.21 aB ± (0.02)
	3	78.92 aA ± (0.42)	6.19 bB ± (0.02)
	7	76.89 bA ± (0.16)	6.06 cB ± (0.05)

n = 3

a-c = means in the same column for the storage period bearing different small letters are: Significantly different ($p < 0.05$)

A – C =means in the same column within each treatment group bearing different capital letters are: